ABSTRACTS (MASTER THESIS)

Biosynthetic enzymes involved in naphthalene ring formation in *Lithospermum erythrorhizon*

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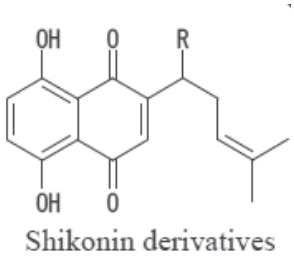
Higher plants produce a large number of secondary metabolites, many of which are applied for medicines, pesticides, spices, dyes, and fragrance etc. These compounds generally have complicated structures exhibiting chirality, and therefore chemical synthesis to supply these natural compounds is not cost effective in many cases. Plant cell culture system provides an effective method for stable production of valuable natural compounds. Application of plant cell cultures for production of secondary metabolite is suitable for high value compounds having a large market size, such as anticancer drugs, and/or those rarely obtained from natural sources due to the scarcity of the plants. The first example of industrial production of such a secondary metabolite by plant cell cultures was achieved in shikonin production by *Lithospermum erythrorhizon* in 1980s.

L. erythrorhison Sieb. et Zucc. (Boraginaceae) is a perennial herbaceous plant that is, at least in recent years, rarely found in Japan, Korea, and China. Roots of this plant appear red-purple, as its root bark (cork layers) contains a large amount of red naphthoquinone pigments, shikonin derivatives (Fig.1).



Shikonin derivatives

Compound	R
Deoxyshikonin	-H
Shikonin	-OH
Acetylshikonin	$-OCOCH_3$
Isobutylshikonin	$-OCOCH(CH_3)_2$
β,β-Dimethylacrylshikonin	$-OCOCH{=}C(CH_3)_2$
Isovalerylshikonin	$-OCOCH_2CH(CH_3)_2$
α-Methyk-n-butylshikonin	$-OCOCH(CH_3)CH_2CH_3$
β-Hydroxyisovalerylshikonin	$-OCOCH_2C(OH)(CH_3)_2$

Fig. 1. Shikonin derivatives detected in *L. erythrorhizon*

The cultured cells of this plant are capable of producing a high amount of shikonin derivatives in M9 medium. Its biosynthesis has been well studied on molecular level so far, while the reaction step of naphthalene ring formation from a key intermediate geranylhydroquinone is still unknown. Taking the advantage that the shikonin production is strictly inhibited by light, a subtractive hybridization experiment was done to identity enzymes responsible for the formation of naphthalene ring structure. Several genes coding for various oxidoreductases were found as being dark-inducible genes. Heterologous expression systems of the candidate clones were established with various host organisms, e.g, tobacco BY2 cells.

[1] Mizukami H, Konishima M, Tabata M, "Variation in pigment production in *Lithospermum erythrorhizon* callus cultures," *Phytochemistry*, vol. 17, pp. 95-97, 1978.

[2] Fujita Y, Hara Y, Suga C, Morimoto T, "Production of shikonin derivatives by cell suspension cultures of *Lithospermum erythrorhizon*. 2. A new medium for the production of shikonin deivatives," *Plant Cell Rep*, vol. 1, pp. 61-63, 1981.

[3] Yamamoto H, Zhao P, Yazaki K, Inoue K, "Regulation of lithospermic acid B and shikonin production in *Lithospermum erythrorhizon* cell suspension cultures." *Chem. Pharm. Bull.*, vol. 50, pp. 1086-1090, 2002.